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http://www.bioblast.at/index.php/MiPNet18.09_IOC80

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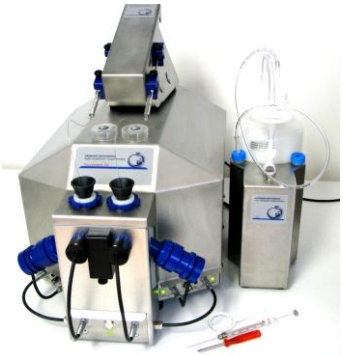
80th International Workshop on HRR and O2k-Fluorometry

2013 October 09-14

Schröcken, Vorarlberg, Austria



The **80th Workshop on High-Resolution Respirometry (HRR)** is the **30th** International Oxygraph Course held in Schroecken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluorescence LED2-Module**, with real-time analysis by **DatLab** and applications of the **TIP2k**. A demo experiment illustrates the princple and shows the unique advantage of simultaneous monitoring of oxygen concentration, respiration and hydrogen peroxide production. Yeast cells are used as a biological reference material which can be obtained world-wide as freeze dried samples.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. In the evenings, general mitochondrial topics are covered; abstracts and experimental experiences are presented by participants.

IOC participants asked invariably for a detailed discussion of protocol design. The **Blue Book** provides a basic introduction to mitochondrial physiology and is complemented by overview persentations with examples, including **DatLab Analysis** of demo files. **Instrumental quality control** is a fundamental component of HRR and will be put to the practical test in teams using six O2k (12 chambers). **O2k-MultiSensor** and particularly O2k-Fluorometry has become an integral part of the O2k-Workshop. Optimization of protocol design for various MultiSensor applications helps to critically evaluate basic principles of mitochondrial physiology. You will also see the **Titration-Injection microPump TIP2k** with feedback-control in action and practice its simple and automatic operation.

Lunch breaks provide an opportunity for relaxing walks and talks, enjoying the refreshing scenery of the secluded alpine environment, offer a visit to the Alpmuseum, or give sufficient spare time for individual practice.

Lecturers and tutors

Gnaiger Erich
Laner Verena
Fontana-Ayoub Mona
Krumschnabel Gerhard

The Universe of Mitochondrial Physiology in 2 ml

Programme IOC80

1 Wednesday, Oct 09

*printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; approx. 1 hour bus drive to Schröcken and Hochtannberg (Salober). Transfer/walk to Hotel Körbersee	IOC-travel
18:30	*Welcome reception at Hotel Körbersee*	Schroecken
19:00	*Dinner*	
20:30-21:00	**Get-together:** introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	IOC80*

2 Thursday, Oct 10

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
	Principles of high-resolution respirometry and O2k-Fluorometry - from switching on the Oxygraph-2k to the experimental result		Gnaiger 2008 POS*
08:30-09:15	**Get O2k-Connected with OROBOROS:** a guided tour to the Oxygraph-2k		get O2k-Connected
09:15-10:00	**Introduction to a DemoExperiment with the O2k**		Pesta 2012 Methods Mol Biol*
10:00	*Coffee / Tea*		
10:30-12:00	**O2k-Demo experiment 1:** Respiration of intact cells and on-line DatLab Analysis: Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (O2k-Fluorescence LED2-Module)		MiPNet18.06_Amplex-Yeast*
12:00	*Lunch - Walk & Talk*		
15:00-16:00	**O2k instrumental setup and sensor service** - overview		O2k-Manual
16:00	*Coffee / Tea*		
	O2k instrumental setup	**OroboPOS service**	
16:30-17:15	Groups 1-5	Groups 6-10	O2k-Start
17:15-18:00	Groups 6-10	Groups 1-5	POS Service
18:30	*Dinner*		
20:00-21:00	**Protocol design: mt-prep - tissues, limitations imposed by fluorometry - controls parallel to fluorometry**		MiPNet18.05 Amplex-Mouse-heart

IOC 80

3 Friday, Oct 11

	Workshop 2	*Weblink*
07:30-08:30	*Breakfast*	
08:30-09:15	**Experimental design 1:** Coupling control protocol with intact cells: ROUTINE, LEAK, ETS, ROX	Cells: PCP
09:15-10:00	**Experimental design 2:** Substrate and coupling control of mitochondrial respiration - MitoPathways through CI+II	The Blue Book pp 29-43*
10:00	*Coffee / Tea*	
10:30-12:00	**SUIT protocol with DatLab Analysis and guide through Excel templates**	DatLab Flux Analysis
12:00	*Lunch – Fishing*	The Blue Book p 56*
15:00-16:00	**Tissue homogenate preparation:** The PBI-Shredder	MiPNet17.03 Shredder vs Fibres*
16:00	*Coffee / Tea*	
16:30-18:00	**O2k-Demo experiment 2:** Respiration with tissue homogenate: SUIT protocol, oxygen regime, washing	Krumschnabel 2013 Abstract MiP2013: 26-27*
18:30	*Dinner*	
20:00-21:00	**Hot MiP-Topics:** 10+5 min presentations of abstracts by participants	IOC80 Abstracts MiPNet18.09*

4 Saturday, Oct 12

	Workshop 3	*Weblink*
07:30-08:30	*Breakfast*	
08:30-09:15	**DatLab O_2 flux analysis:** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
09:15-10:00	**DatLab Guide through the menus:** DL-Demo files and DL-Excel templates	DatLab Guide
10:00	*Coffee / Tea*	
10:30-12:00	**DatLab Analysis: hands-on in 10 teams**	DatLab Flux Analysis
12:00	*Lunch - Walk & Talk*	
15:00-16:00	**Instrumental quality control 1:** **The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.**	O2k-Calibration
16:00	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
16:30-17:15	**Instrumental quality control 2:** **O2k-Background test and on-line analysis of oxygen flux.**	O2k-Background*
17:15-18:00	**Hands-on (6 groups): O2k-Background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 - 200 µM.** **O2k-Background with automatic TIP2k or manual titrations.**	O2k-Background*
18:30	*Dinner*	

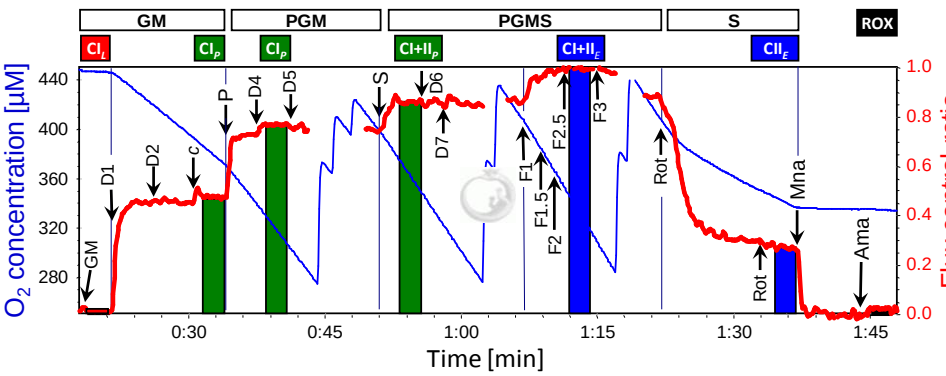
20:00-21:00	**10x5-min team presentations: DatLab Analysis: results, what did we learn, what was difficult, open questions?**

5 Sunday, Oct 13

	Workshop 4	*Weblink*
07:30-08:30	*Breakfast*	
08:30-09:15	**O2k-MultiSensor overview and O2k-Fluorometry applications:** Amplex™ red and safranin	MiPNet17.17_Amplex-Mouse-brain*
09:15-10:00	**OXPHOS analysis: A challenge for the simultaneous measurement of respiration and mt-membrane potential**	
10:00	*Coffee / Tea*	
10:30-12:00	**O2k-Demo experiment 3: Respiration and steady-state feedback control of oxygen levels with the TIP2k.**	TIP2k User Manual
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum: Guided tour and reception: 15 €*	www.alpmuseum.at*
16:00	*Coffee / Tea*	
16:00-16:45	**Working groups: Elaborate answers to the 'Questions for the O2k-Workshop'** *(study IOC-Questions in advance!)*	IOC-Questions*
16:45-17:15	**IOC-Questions - discussion of 'Answers'**	
17:15-18:00	**Introduction to trouble shooting**	O2k-Troubleshooting
18:00-18:45	**The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science**	www.bioblast.at
19:00	*Dinner*	
20:30-21:00	**Panel Discussion - Feedback IOC80**	O2k-Feedback
	Farewell party	

6 Monday, Oct 14

	Departure / Fish project	
	Breakfast	
	Early morning: Departure / Fish heart and liver	Gnaiger 1993 Verh Dtsch Zool Ges*



SUIT protocol with trout heart homogenate in a high oxygen concentration regime (MiR06Cr, 15 °C, reox with H_2O_2) (Krumschnabel 2013 Abstract MiP2013)*.

Participants

Name	Lab
Bagur Quetglas Rafaela	**FR_Grenoble_Schlattner U:** Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Joseph Fourier, Grenoble. - metabolic control analysis, respiration regulation, respiratory chain complexes activities.
Becker Christina	**DE_Cologne_Trifunovic A:** Institute of Genetics, University of Cologne.
Consitt Leslie	**US_OH Athens_Consitt L:** Biomedical Sciences, Ohio University. - Diabetes, obesity, aging, skeletal muscle.
Feng Jianhua	**CH_Zurich_Zaugg K:** Universitätsklinik für Radio-Onkologie, Inselspital, Bern. - p53, mitochondria, CPTIC
Fernandez-Vizarra Erika	**UK_Cambridge_Zeviani M:** MRC Mitochondrial Biology Unit - Cambridge. - Mitochondrial Respiratory Chain assembly disorders, Complex I, Complex III, Complex IV.
Ferrando Miguel Rosa	**FR_Paris_Bigou S:** Hôpital de la Pitié-Salpêtrière, Paris. - Respirometry in cell culture and animal models in Parkinson's disease
Fontana-Ayoub Mona (tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**
Garcia-Souza Luiz Felipe	**BR_Rio de Janeiro_Oliveira MF:** Institute of Medical Biochemistry, Federal University of Rio de Janeiro. - platelet, thrombin, activation
Gnaiger Erich (lecturer)	**AT_Innsbruck_OROBOROS INSTRUMENTS**: DSL, Dept Visceral, Transpl Thoracic Surgery, Medical University Innsbruck.
Garrabou Gloria	**ES_Barcelona_Moren C:** Muscle Research and Mitochondrial Function Laboratory, University of Barcelona.
Goy Christine	**DE_Duesseldorf_Haendeler J:** IUF-Leibniz Research Institute for Environmental Medicine/Molecular Aging Research. - Mitochondrial Telomerase Reverse Transcriptase, mitochondrial functions
Jung Ki-Duck	**KR_Seoul_Mymed:** Mymed Co. Ltd, Seoul.
Kobayashi Hirosuke	**JP_Kanagawa_Kobayashi H:** Dept. of med. Engineering and Technology, Kitasato University School of Allied Health Sciences.
Kokubo Kenichi	**JP_Kanagawa_Kobayashi H:** Dept. of med. Engineering and Technology, Kitasato University School of Allied Health Sciences.
Krumschnabel Gerhard (tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**
Krylova Tatiana	**RU_Moscow_Zakharova E:** Research centre for medical genetics, RAMS, Moscow. - Point mutations of mitochondrial DNA.
Kwak Hyo-Bum	**KR_Incheon_Kwak HB:** Dept. of Kinesiology, Inha University, Incheon.
Laner Verena (tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**
Michalak Slawomir	**PL_Poznan_Michalak S:** Department of Neurochemistry and Neuropathology, Poznan University. - neurological paraneoplastic syndromes, multiple sclerosis, Parkinson's disease, peripheral blood mononuclear cells
Mäkelä Kari	**FI_Oulo_Mäkelä K:** Institute of Biomedicine/Physiology University of Oulu. - brown adipose tissue, white adipose tissue
Meszaros Andras	**HU_Szeged_Boros M:** Institute of Surgical Research, School of Medicine, University of Szeged. - hypoxia, reoxygenation, electrophyl methyl groups (EMGs), methane, long-chain fatty acids

Mukhina Irina	**RU_Nizhny Novgorod_Mukhina I:** Central Research Laboratory, Nizhny Novgorod State Medical Academy. - Cell respiration, hypoxia, oxidative stress, Ischemia-reperfusion injury, mitochondrial pathways, respiratory control
Salin Karine	**UK_Glasgow_Metcalfe N:** Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow. - fish, oxidative stress, environmental response, coupling.
Scheidnagl Simone	**AT_Innsbruck_Gnaiger E**: Institute of Zoology, Innsbruck University
Schreiber Renate	**AT_Graz_Zechner R:** Institut für Molekulare Biowissenschaften - Universität Graz. - Adipose tissue
Shelchkova Natalia	**RU_Nizhny Novgorod_Mukhina I:** Central Research Laboratory, Nizhny Novgorod State Medical Academy. - Cell respiration, hypoxia, oxidative stress, Ischemia-reperfusion injury, mitochondrial pathways, respiratory control
Siddall Charles Parker	**US_IN Indianapolis_Brozinick JT:** Eli Lilly and Company, Lilly Corporate Center.
Stephens Natalie	**US_FL Orlando_Sparks LM:** Translational Research Institute for Metabolism and Diabetes, Orlando.
Van Heck-Kappen Antonia	**NL_Nijmegen_Rodenburg R:** Department Laboratory Medicine, Radboud University Nijmegen Medical Centre. - Diagnostics of Human mitochondrial disorders in skeletal muscle and fibroblasts by measuring ATP production, oxidation rates and enzyme activity from respiratory chain
Willmes Diana	**DE_Berlin_Birkenfeld AL:** Center for Cardiovascular Research (CCR), Department of Endocrinology, Diabetes and Nutrition, Charité Berlin. - metabolism, hepatocytes, obesity.
Wintjes Liesbeth	**NL_Nijmegen_Rodenburg R:** Department Laboratory Medicine, Radboud University Nijmegen Medical Centre. - Diagnostics of Human mitochondrial disorders in skeletal muscle and fibroblasts by measuring ATP production, oxidation rates and enzyme activity from respiratory chain
Wuest Rob C	**NL_Amsterdam_Wuest RC:** Department of Physiology VU Medical Center. - cardiomyocytes, diabetes, heart failure, ROS
Volodina Maria	**RU_Moscow_Skulachev VP:** Research Center for Obstetrics, Gynecology and Perinatology, Moscow. - mitochondria, oxidative stress, pregnancy disorders, fertility

MiPNet18.07 Abstracts IOC80: 10+5 min

Hot topics in Mitochondrial Physiology

Fernandez-Vizarra E: Testing OXPHOS biogenesis and function in mitochondrial disease models.

Our laboratory is mainly interested in the discovery of new genes whose mutations cause oxidative phosphorylation (OXPHOS) defects in human tissues, leading to mitochondrial disease syndromes. Once a candidate mutant gene is identified, usually by linkage or NGS analysis, our aim is to validate the pathogenic role of the mutation and understand the molecular mechanism linking the variant protein to faulty OXPHOS and disease. This goal can be achieved by studying tissue samples obtained from the patients, usually muscle biopsies and cultured fibroblasts from skin biopsies. Additionally, knocked-down expression of the candidate gene product can be achieved by

RNAi technology applied to cultured cell lines. Furthermore, mouse knock-out models for the gene of interest can eventually be generated to gain deeper understanding of the biochemical and pathophysiological effects associated with the lack of the protein in living tissues, and whole organism. To test OXPHOS functionality in this wide spectrum of tissues and cells from patients as well as in the ad hoc recombinant models, we will perform oxygen consumption measurements in basal conditions and on exposure to specific substrates and inhibitors. This first characterization will provide key information to then continue with the analyses of physical status of OXPHOS-related complexes and their individual activities.

Garcia-Souza LF: Thrombin trigger mitochondrial functional remodeling in human platelets.

Evidence has indicated that pro-coagulant factors modulate platelet energy and redox metabolism pathways. However, the involvement of mitochondria during platelet activation remain poorly understood and was investigated in the present work.

Human platelets were collected from healthy volunteers, isolated in M199 medium, and subsequently challenged with different thrombin concentrations. Several parameters were analyzed in activated platelets such as P-selectin externalization, respiration, lactate and nitric oxide (NO) production. The assessment of oxygen flow and metabolic states in platelets was carried out in the presence of several mitochondrial modulators by titrating each one of them in the following order: Oligomycin, FCCP, Rotenone and Antimycin A in the presence or absence of thrombin.

During platelet activation, CD62p expression increased and was followed by an increase in lactate secretion and NO production with high correlation between each other. Analyzing mitochondrial states we could detect changes in several states, mainly ROUTINE, OXPHOS, proton leak and reserve capacity. However, normalizing our signal by the highest uncoupled oxygen consumption (ETS), we detected differences in residual oxygen consumption (ROX) alongside other states.

Our data indicate that mitochondria from human platelets are affected by their ativation. This raises the concern of using platelets as models of indirect studies such as mitochondrial diseases and Alzheimer. The mitochondria play a very important role in platelet physiology and is not an inert within the cell, is affected by a number of substances that are present at the time of activation, such as calcium and nitric oxide. Therefore, it is necessary to evaluate the changes in mitochondrial human platelets against different agonists and inhibitors of coagulation.

Wuest RC: Bioenergetics and mitochondrial dysfunction in chronic heart failure.

In chronic heart failure, alterations occur in cardiac metabolism, enzyme content calcium handling and mitochondrial function. Mitochondrial dysfunction is a common observation in cardiac tissue in patients with left and right-ventricular heart failure, ischemic reperfusion injury, cardiac preconditioning, mechanical ventilation and type II diabetes. This suggest a general role for mitochondria in the cellular pathophysiology of these diseases. Despite the importance of mitochondria as regulators of energy supply, little is known on the processes in vivo that determine the activation of mitochondrial respiration in health and disease.

A photometry-based technique was used to simultaneously measure contractile function and autofluorescence of NADH (reduced nicotinamide adenine dinucleotide) and FAD (oxidized flavin adenine dinucleotide) in thin cardiac trabeculae. Three groups were studied: CH (monocrotaline: 40 mg.kg-1) or HF (60 mg.kg-1) and CON (saline). After 23±1 days, right ventricular trabeculae were excised, attached to a force transducer and superfused with oxygenated tyrode with 1 mM Ca2+. Autofluorescence of both NADH (excitation: 340 nm; emission: 470 nm) and FAD (450/525 nm) were recorded using an inverted microscope during transitions in pacing frequency between 0.5 and 1.2 or 3 Hz at 27 °C. Despite a smaller increase in force-time-integral, the initial changes in NADH and FAD were less pronounced in CH and HF than in CON, indicating a mismatch between dehydrogenase activity and mitochondrial respiration. These data reveal an altered mitochondrial complex function in CH and HF and further experiments with the Oroboros are planned to fully understand the changes in bio-energetic function in chronic heart failure.

Accommodation and Location

Hotel Körbersee www.koerbersee.at
T +43 5519 265; hotel@koerbersee.at

More detail?

O2k-Manual – www.oroboros.at/?O2k-Manual
O2k-Protocols – www.oroboros.at/?O2k-Protocols
>1,000 O2k-Publications – www.bioblast.at/index.php/O2k-Publications

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www.oroboros.at/?MitoCom-Tyrol

Contact

Erich Gnaiger, PhD
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria
www.bioblast.at

OROBOROS INSTRUMENTS Corp
Schöpfstrasse 18
A-6020 INNSBRUCK, Austria
T +43 512 566796 F +43 512 566796 20
Email instruments@oroboros.at
Homepage: www.oroboros.at
Cooperation and Feedback in Science